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The Role of GABA in the Anticonflict Action of Sodium Valproate and Chlordiazepoxide

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VELLUCCI, S. V. AND R. A. WEBSTER. *The role of GABA in the anticonflict action of sodium valproate and chlordiazepoxide*. PHARMACOL BIOCHEM BEHAV 21(6) 845–851, 1984.—The anxiolytic effects of chlordiazepoxide (CDP) in the Geller-Seifter rat conflict test have been compared with those of muscimol and sodium valproate in order to evaluate the possible role of GABA in the anxiolytic action of the benzodiazepines. The anticonflict activity of CDP was inhibited by the benzodiazepine antagonist Ro15-1788, as well as by bicuculline and picrotoxin. The weaker anticonflict effect of muscimol was overcome by both bicuculline and picrotoxin. Sodium valproate had a marked anticonflict effect which was only inhibited by picrotoxin. Thus, while the anxiolytic action of CDP depends on GABA function in some way, it can be distinguished from the effects of muscimol and sodium valproate which appear to act at the GABA receptor and the chloride ion channel, respectively.

GABA Geller-Seifter conflict test Sodium valproate Chlordiazepoxide

IT is well established that benzodiazepines are capable of augmenting GABA function in the central nervous system [8, 18, 37] and indeed this hypothesis can adequately explain some of their pharmacological actions, in particular their anticonvulsant and muscle relaxant properties. On the other hand, their anxiolytic effects may be related to changes in the functional activity of central, 5-hydroxytryptamine (5HT) systems [7, 12, 39, 48, 53], although it is likely that these are secondary to changes in GABA function (perhaps by inhibiting 5HT release). Nevertheless, it has not been easy to link the anxiolytic effects with changes in GABA function. Some authors [4,46] have shown that GABA antagonists such as bicuculline and picrotoxin can reduce the anticonflict effects of benzodiazepines, although it has been suggested that the effects of picrotoxin may have been non-specific [43]. On the other hand directly or indirectly acting GABA-mimetics have either no effect [7,11] or have an anxiolytic profile [6,17].

In the work described here, we have compared the effect in the Geller-Seifter rat conflict test [14], of chlordiazepoxide (CDP) with some directly and indirectly acting GABA mimetic compounds and their antagonists, in an attempt to investigate further whether or not GABA-ergic mechanisms are involved in mediating the anxiolytic properties of the benzodiazepines.

METHOD

Animals and Surgery

Male Lister hooded rats (home-bred) 180–220 g were housed singly in a room maintained at 22°C with an 11 hr light:13 hr dark cycle (lights on at 07.00 hr). For direct injection of substances into the CNS, the animals were anaesthetized with halothane and a permanent indwelling stainless steel cannula (o.d. 1.0 mm) was placed stereotaxically into the right or left lateral cerebral ventricle (50% for each). (Stereotaxic co-ordinates: 0.9 mm posterior, and 1.5 mm lateral to the bregma, 4 mm below the skull surface.) Each cannula was held in position with acrylic surgical cement (Simplex) set onto 3 small screws in the skull. The animals were allowed to recover from the surgery for 2 weeks prior to training, during which time food and water were freely available.

Apparatus

The animals were tested in a standard rodent operant test chamber (25×25×23 cm; Campden Instruments Ltd.). The floor of the chamber consisted of 16 stainless steel bars (0.48 cm diameter) spaced 1.0 cm apart, through which a scrambled electric shock could be delivered. The test-chamber contained two stainless steel levers (3.75 cm wide) projecting

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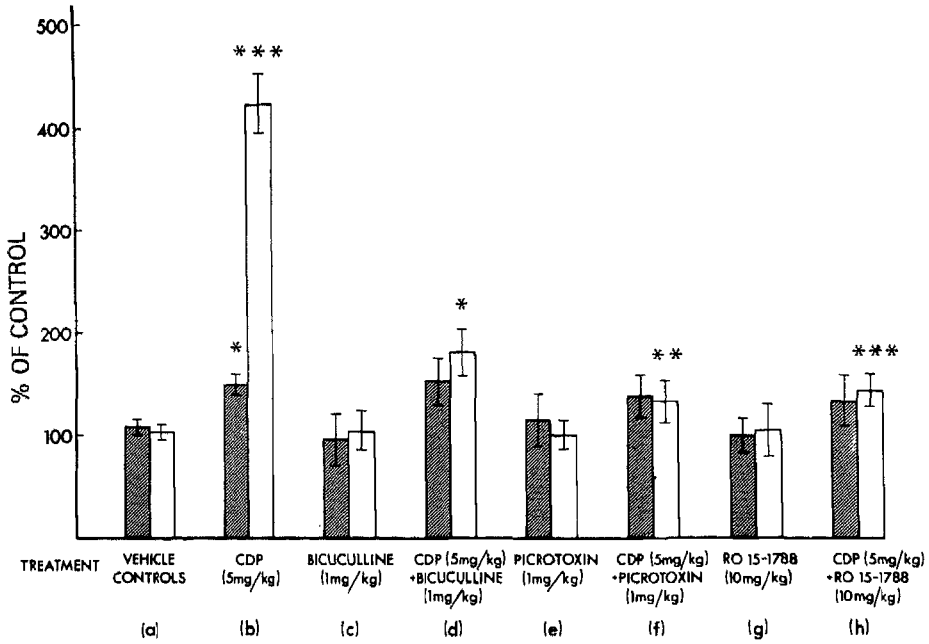

FIG. 1. Modification of the effects of CDP (5 mg/kg, IP, 30 min before testing) on unpunished (shaded column) and punished (open column) responding, by bicuculline (1.0 mg/kg), picrotoxin (1.0 mg/kg) and Ro15-1788 (10 mg/kg), administered 15, 20 and 15 min after CDP, respectively. The ordinate shows the response rate as a percentage of the corresponding untreated control response, where the latter is 100%. Drug treatments are indicated on the abscissa. Each value represents the mean (±SEM) obtained from 10 animals. Statistically significant from corresponding control groups: $*p<0.05$; $**p<0.005$; $***p<0.001$ (Paired t-test).

2.5 cm into the chamber from one side and 6.0 cm from the grid floor. Reinforcement was provided in the form of 45 mg precision food pellets (Campden Instruments Ltd.) which were delivered by a dispenser to the magazine tray, situated midway between the two levers. The chamber was supplied with 5 lights (each 2.8 W, 24 V). One was situated in the centre of the ceiling; 3 on the front panel above the magazine tray and both levers, respectively, and one in the food magazine itself. The entire test-chamber was enclosed in a light-proof, sound attenuating ventilated box so that the animals could not be distracted by external events but could be viewed through a darkened window. A modified Geller-Seifter conflict test was used [14,54]. Animals were trained to press the left-hand lever on a continuous reinforcement schedule, with the only illumination being the magazine light. The schedule was then gradually increased to a fixed ratio-21 (FR-21) schedule. Once a stable response rate had been established, 6 min FR-21 periods, alternating with 3 min periods of continuous reinforcement (CRF) were introduced. During the CRF period all the chamber lights were on. The total test time was 27 min and consisted of one FR-21 portion (6 min) alternating with one CRF portion (3 min), repeated three times. Finally, scrambled footshock, applied via the grid floor, was introduced during all the CRF periods. The shock intensity, initially 0.1 mA, was gradually increased by 0.05 mA until a stable but low response rate was achieved during each CRF period. The range of shocks finally used was 0.3–0.7 mA and these did not affect the response rate during the FR-21 portions of the test. Only those animals which showed stable and reproducible response rates during the 2 components of the test were used for drug treatments. The animals were tested once daily (Mon-Fri) at the same time of day and were only allowed access to food during the test period and for a period of 1 hr immediately after testing. On those days when the animals were not tested they received a pre-determined quantity of food. Each animal was used as its own control, with control trials being carried out before and after each drug or vehicle-treatment trial. No animal received drugs more than twice per week. The groups of animals that were finally used in the experiments were "drug-experienced" and had been treated on 3–4 separate occasions with an effective dose of CDP (5 mg/kg, IP) firstly to ascertain that the animals were responsive to the anticonflict effects of CDP and secondly to allow for the so-called "initial treatment" phenomenon in which there is an initial progressive increase in the anticonflict effect of CDP, as described previously by several investigators [7,32].

Drugs

Chlordiazepoxide hydrochloride (CDP; Roche Products Ltd.) was dissolved in distilled water and injected intraperitoneally (IP) in a dose of 5 mg/kg (5 mg/ml) 30 min prior to testing.

Ro15-1788 an imidazodiazepine derivative (ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo-[1,5-a]benzodiazepine-3-carboxylate: Roche Products Ltd.) was suspended in distilled water to which Tween 80 had been added (2 drops/10 ml) and injected IP in doses of 5, 10, 50 or 100 mg/kg (1 ml/kg) at various times prior to testing.

(+) Bicuculline (Sigma Chemical Co.) was dissolved in a minimum quantity of 0.5 N HCl, and the volume made up to 10 ml with distilled water. Bicuculline was administered IP, in doses of 0.3, 0.5 or 1 mg/kg (1 ml/kg) 15 min prior to testing.

Picrotoxin (Sigma Chemical Co.) was suspended in saline

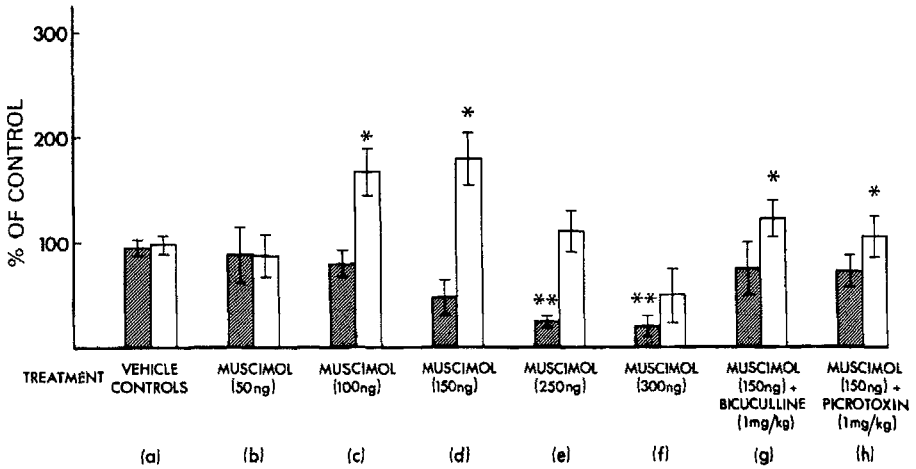

FIG. 2. Modification of the effect of muscimol (150 ng, ICV, 15 min before testing) on unpunished (shaded column) and punished (open column) responding by bicuculline (1.0 mg/kg, IP) and picrotoxin (1.0 mg/kg, IP) administered at the same time as, and 5 min after muscimol, respectively. The effect of various doses of muscimol injected ICV 15 min before testing is also shown (b–f). The ordinate shows the response rate as a percentage of the corresponding untreated control response, where the latter is 100%. Drug treatments are indicated on the abscissa. Each value represents the mean (±SEM) obtained from 10 animals. Significantly different from corresponding control groups: $*p<0.05$; $**p<0.001$ (Paired t-test).

to which Tween 80 (2 drops/10 ml) had been added. Picrotoxin was injected IP, in a dose of 1 mg/kg) 10 min before testing.

Sodium valproate (Labaz, Stockport, Cheshire) was dissolved in saline and administered IP in doses of 100, 200 or 300 mg/kg (1 ml/kg) 5 min before testing.

Aminooxy-acetic acid (AOAA: Sigma Chemical Co.) was dissolved in saline and administered IP in doses of 6.25, 12.5, 25 and 50 mg/kg (1 ml/kg), 30 min prior to testing.

Muscimol (Fluka A.G.) was dissolved in artificial cerebrospinal fluid (ACSF) and administered intracerebroventricularly (ICV) in 10 μl volumes, containing 50, 150, 250 or 300 ng muscimol, 15 min prior to testing.

Control treatments consisted of equal-volume injections of the appropriate vehicle (i.e., 1 ml/kg for IP and 10 μl for ICV administration). For each group of 10 animals drug-treatments were given in varying sequences to minimize order effects. At the end of the series of experiments the animals were killed and, where necessary, the cannulae placements verified.

RESULTS

Initially, a stable rate of punished responding was established with continuous reinforcement (CRF) which was equivalent to 15.2±2.4% of the response rate observed during CRF in the absence of punishment.

The results for each drug or vehicle-treatment were expressed as a percentage of the mean of 3 control responses obtained on 2 consecutive days prior to, and on the day after treatment. The results for punished and non-punished periods are shown separately. Statistical significance was determined by using a 2-tailed t-test for paired observations, with each animal acting as its own control.

The mean rates of lever pressing in the absence of drug or vehicle treatments were 453±50 and 12.1±1.2 for the non-punished (FR-21) periods (18 min) and punished (CRF) periods (9 min), respectively.

The IP administration of the vehicles or the ICV administration of ACSF had no significant effects on either of the response rates (Figs. 1a, 2a, 3a).

Chlordiazepoxide

Chlordiazepoxide 5 mg/kg, IP administered 30 min prior to testing produced a slight, but significant increase ($p<0.05$), in the rate of unpunished responding and a very marked increase in the rate of punished responding ($p<0.001$) (Fig. 1b). Bicuculline alone in doses of up to 1.0 mg/kg IP administered 15 min prior to testing had no effect on either of the response rates (Fig. 1c). However, bicuculline (1.0 mg/kg) administered 15 min after CDP (i.e., 15 min before testing) caused a significant ($p<0.05$) reduction in the rate of punished responding that is normally obtained after CDP, without affecting the rate of unpunished responding (Fig. 1d). Similarly picrotoxin in a dose of 1 mg/kg administered 10 min before testing had no significant effect on the two response rates (Fig. 1e). On the other hand this dose of picrotoxin significantly ($p<0.005$) reduced the rate of punished responding normally obtained after CDP without significantly affecting the unpunished response rate (Fig. 1f). The benzodiazepine antagonist, Ro15-1788 (10 mg/kg), administered 15 min before testing, did not affect either of the response rates (Fig. 1g), compared with (Fig. 1a). Indeed Ro15-1788 alone in doses of up to 50 mg/kg IP, administered 5, 15 and 30 min before testing had no significant effects on either of the response rates (unpublished). However, when Ro15-1788 (10 mg/kg) was administered 15 min before testing, but 15 min after CDP, it caused a significant ($p<0.001$) reduction in the rate of punished responding normally obtained with CDP without significantly affecting the unpunished response rate (Fig. 1h).

Aminooxy-Acetic Acid

Aminooxy-acetic acid (a GABA-T inhibitor) in a dose of

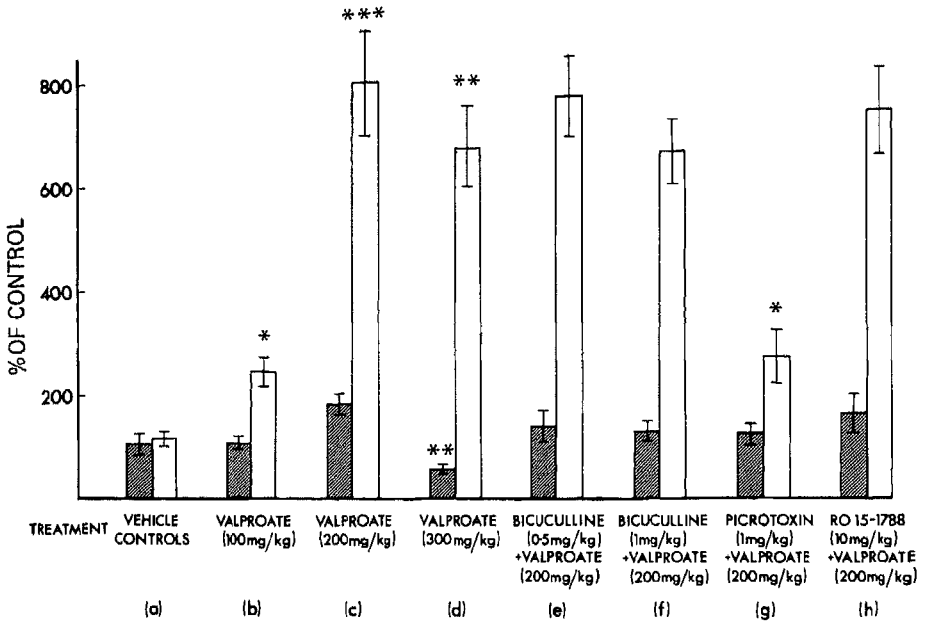

FIG. 3. Modification of the effects of sodium valproate (200 mg/kg, IP, 5 min before testing) on unpunished (shaded column) and punished (open column) responding by bicuculline (0.5 and 1.0 mg/kg, IP), picrotoxin (1.0 mg/kg, IP) and Ro 15-1788 (10 mg/kg, IP) administered 10, 5 and 10 minutes before valproate, respectively. The effects of various doses of valproate administered 5 min before testing are also shown (b–d). The ordinate shows the response rate as percentage of the corresponding untreated control response, where the latter is 100%. Drug treatments are indicated on the abscissa. Each value represents the mean (±SEM) obtained from 10 animals. Significantly different from corresponding control groups: *$p<0.05$; **$p<0.005$; ***$p<0.001$ (Paired t-test).

6.25 mg/kg IP, administered 30 min prior to testing did not significantly affect the response rates (89.7±9.2% and 112.6±12.0% of control rates for punished and unpunished responding, respectively). Higher doses of AOAA (12.5, 25 and 50 mg/kg) produced sedation and depression of both response rates (data not shown).

Muscimol

Muscimol (100 and 150 ng) administered ICV (Fig. 2c and 2d) produced a significant ($p<0.05$) increase in the rate of punished responding despite depressing unpunished responding. This anticonflict effect was no longer evident with higher doses (250 and 300 ng) of the compound (Fig. 2e and 2f). In fact with the highest dose of muscimol (300 ng) there was a marked decrease in the rate of punished responding ($p<0.001$, compared with the corresponding controls). This was due to behavioural depression which was reflected in the dose-related decrease in the rates of unpunished responding after treatment with 100, 150, 250 and 300 ng muscimol. Bicuculline and picrotoxin significantly ($p<0.05$) reduced the anticonflict effects of a test-dose (150 ng) of muscimol and prevented the marked decrease in unpunished responding which is also observed with this dose of muscimol (Fig. 2g and 2h, respectively).

Sodium Valproate

Sodium valproate (100 and 200 mg/kg IP, 5 min prior to testing) produced progressive increases in the rates of punished responding (Fig. 3b and 3c; $p<0.01$ and $p<0.001$, respectively). The rate of unpunished responding was unaffected by the lower dose of valproate and slightly (but not significantly) increased by the 200 mg/kg dose. A higher dose of valproate (300 mg/kg) also significantly ($p<0.005$) increased the rate of punished responding. However, the anticonflict effect seen with this dose was not significantly different from that observed with the 200 mg/kg dose and it also significantly ($p<0.005$) reduced the rate of unpunished responding (Fig. 3d). Bicuculline, in doses of 0.5 and 1.0 mg/kg IP given 15 min before testing, 10 min before sodium valproate (200 mg/kg), failed to significantly affect the response to this compound (see Fig. 3e and 3f). On the other hand picrotoxin 1 mg/kg, administered 10 min before testing, 5 min before valproate (200 mg/kg), significantly ($p<0.05$) reduced the rate of punished responding normally obtained after this dose of valproate. The rate of unpunished responding was slightly, but not significantly, reduced by this dose of picrotoxin (Fig. 3g). Ro15-1788 (5 and 10 mg/kg) administered 15 min before testing (10 min before sodium valproate (200 mg/kg), failed to alter the response normally obtained with this dose of valproate (Fig. 3h).

DISCUSSION

Our observation that the anticonflict effects of CDP could be reduced by bicuculline or picrotoxin is in agreement with the findings of several authors who have shown that the behavioural actions of the benzodiazepines can be antagonized by substances that block GABA receptors or GABA synthesis [4, 43, 46, 55]. Some authors have also reported that picrotoxin could significantly depress the rate of unpunished responding, but there was no evidence of this effect with the low doses of picrotoxin used in the present work. The anticonflict effect of CDP was also overcome by the imidazodiazepine derivative, Ro15-1788, see also [22,54]. This compound is a potent and specific inhibitor of [^{3}H]

diazepam binding to rat brain synaptosomal fractions and is capable of antagonizing most of the centrally-medidated effects of active benzodiazepine derivatives [22]. Ro15-1788 has also been reported to abolish the immobility and sedation produced by suitable doses of sodium valproate, to antagonize some of the electrophysiological effects of valproate and to have a short-lasting antagonist effect on valproate when tested in a condensed form of the conflict test [33,34]. Consequently, these authors concluded that the therapeutic effects of valproate could be mediated via an action at benzodiazepine receptors. However, we did not observe any attenuation of the anticonflict effects of valproate by Ro15-1788, even in the initial phases of the conflict test employed here, despite the fact that we used similar drug doses and CRF periods. Furthermore, Ro15-1788 does not affect the valproate-induced increase in seizure threshold to leptazol [35]. Therefore it is considered unlikely that the anticonflict and anticonvulsant effects of valproate are mediated via a direct action at benzodiazepine receptors, and indeed it has been shown that sodium valproate does not alter basal or GABA receptor-stimulated [^{3}H] diazepam binding to rat brain membranes [51]. Similarly the results from binding studies and those obtained with the isolated superior cervical ganglion of the rat [35], suggest that Ro15-1788 does not directly antagonize the effects of GABA, although we did not test the interaction between muscimol and Ro15-1788 in the conflict test.

If an increase in GABA function does play a role in the anxiolytic effect of the benzodiazepines, then it might be expected that aminooxy-acetic acid (AOAA), a GABA transaminase inhibitor which increases cerebral GABA levels, would possess anxiolytic properties. However, we did not find that AOAA had anticonflict properties and there are no reports in the literature to support this idea [7, 38, 53]. Similarly, more "specific" GABA-T inhibitors (e.g., γ-vinyl-GABA and γ-acetylenic-GABA) do not exert anxiolytic effects [44]. Of course an increase in brain GABA levels does not necessarily mean that there is a concomitant increase in the neurotransmitter pool of GABA and indeed the increased brain GABA concentrations produced by AOAA are believed to be associated primarily with non-nerve terminal components (e.g., neural perikarya and glial cells; [23]). Certainly the lack of anticonflict effect observed after AOAA treatment is unlikely to be due to poor absorption from the site of injection, as the higher doses used produced marked ataxia. Nevertheless we were able to show that some assumed GABA mimetic compounds (e.g., muscimol and sodium valproate) can exhibit anticonflict activity and the possibility that GABA mechanisms are involved in their anxiolytic action was supported by the observation that the anticonflict effects produced by muscimol could be reduced by bicuculline and picrotoxin, and those of valproate by picrotoxin.

Other studies of the anxiolytic effects of muscimol have been far from conclusive. When administered IP it was shown to exhibit some anticonflict activity but this action was not dose-related and not thought to be associated with the potentiation of benzodiazepine activity [42, 43, 49, 50]. Since systemically administered muscimol is rapidly metabolized, only approximately 0.02% of such a dose enters the CNS [2,30]; the lack of a dose-related response after systemic administration may, therefore, be due to its rapid metabolism and failure to cross the blood-brain barrier in sufficient amounts. The results reported here show that after ICV administration, a dose-related anticonflict effect can be observed with low doses of muscimol, whereas higher doses produced ataxia. These findings are in agreement with those of other authors who used anxiolytic tests based on the punishment-induced suppression of drinking in the thirsty rat [6,17]. However, when muscimol is given ICV it cannot be assumed that it reaches those regions of the brain which are accessible to systemically administered chlordiazepoxide and sodium valproate, and this could explain some of the observed differences in their effects. Indeed the ataxia produced by muscimol may be consequent upon its concentration in a given brain area while an anticonflict effect could require the more widespread distribution of the drug that could be achieved with systemically administered CDP and sodium valproate. Also the effect of muscimol could be complicated by the fact that even though it binds preferentially to $GABA_A$ rather than $GABA_B$ sites [5], it actually binds to more Na^+-independent sites than GABA itself [10], some of which show binding for glycine and β-alanine [9] and others for benzodiazepines [25].

The anticonvulsant effects of valproate were originally thought to be related to prevention of GABA metabolism by inhibition of GABA-transaminase [31,45] and succinic semialdehyde dehydrogenase [20]. However, a direct correlation between elevation of brain GABA and anticonvulsant activity does not exist for this drug [21], since it is effective against electroshock-induced convulsions within 5 min of administration [41] whereas a significant elevation of cerebral GABA is seen only after 30 min [40]. Valproate can influence GABA function directly and will potentiate GABA-induced conductance increases in cultured mammalian spinal cord neurones [29] and GABA-mediated inhibition of rat cortical neurones [1, 15, 24].

The present results show that sodium valproate is capable of producing a dose-related anticonflict effect, and are in agreement with those of other authors who demonstrated that valproate produced a dose-related anxiolytic effect in various animal tests of anxiety [13, 26, 34, 38] in the absence of concomitant increases in forebrain GABA concentrations [38]. Unlike some of these authors, however, we did not observe any antagonism of the anticonflict effects of valproate by bicuculline and indeed, it is considered unlikely that valproate acts directly on GABA receptors, in a manner similar to bicuculline, as it fails to affect [^{3}H] muscimol binding [28].

Our observation that picrotoxin, which blocks the GABA-regulated chloride ionophore, could antagonize the anticonflict effects of valproate, at doses which did not affect the rate of non-punished responding, is in agreement with the observation that valproate could inhibit the binding of [^{3}H] dihydropicrotoxinin to its receptors with IC_{50} values similar to those of therapeutically effective concentrations of the drug, thus suggesting that valproate may enhance GABA transmission by acting at the dihydropicrotoxinin (picrotoxin) site to prolong the life-time of GABA receptor-regulated chloride ionophores [51]. In support of this idea are the observations that R05-3663 (1,3 dihydro-5-methyl-2H-1,4-benzodiazepin-2-one), a convulsant benzodiazepine derivative, believed to act mainly at the picrotoxin-sensitive chloride ionophore site and not at benzodiazepine receptors [16, 19, 27, 36, 46] could antagonize the electrophysiological, behavioural and anticonflict effects of sodium valproate [33,34].

The finding that a compound such as sodium valproate, which is believed to act at the chloride ionophore, has anxiolytic properties is in agreement with reports that etazo-

late, a pyrazolopyridine derivative is also an anxiolytic [3] acting at the picrotoxin site and not directly on GABA or benzodiazepine receptors [52].

Clearly drugs can produce an anxiolytic effect by augmenting GABA function in a number of ways that are susceptible to different antagonists. While behavioural studies of the type reported here are far removed from synaptic pharmacology, it is important to try to reconcile our results with published concepts of the benzodiazepine receptor/GABA receptor/chloride ionophore complex. Generally these show that the benzodiazepine and GABA receptors, although separate entities, may be linked to chloride channels and that the benzodiazepines can only function in the presence of a functional or activated GABA receptor. The results presented here are in keeping with this concept. Thus if CDP ultimately produces its anticonflict effect by opening chloride channels after initially activating the benzodiazepine receptor but requires the presence of a functional GABA receptor then, as we observed, its effect would not only be blocked by the benzodiazepine receptor antagonist, Ro15-1788, but also by bicuculline and picrotoxin, antagonists at the GABA receptor and chloride ion channel, respectively. In contrast, if sodium valproate directly activates chloride ion channels then its anticonflict activity should only be antagonized by picrotoxin, as observed, while that of the GABA agonist, muscimol will be antagonized by both the GABA receptor and chloride ion channel antagonists. Needless to say this always assumes a specificity of action of the antagonists that may not be entirely justified.

CONCLUSIONS

The data reported here provide evidence that changes in GABA function may be associated with the anxiolytic properties of benzodiazepine derivatives.

It is possible that sodium valproate exerts its potent anticonflict effect by augmenting GABA function via an action at the level of the chloride ionophore, and not at GABA or benzodiazepine receptors.

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